ACTOMYOSIN AND MUSCULAR CONTRACTION

by

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It has been shown in the author's laboratory[10] that two structural proteins can be extracted from the muscle fibril, actin (F. B. STRAUB) and myosin. The two, if mixed at a proper ionic concentration, unite to a complex, actomyosin, which has the remarkable property of contractility. Actomyosin threads contract under influence of ATP. This contraction, though imitating in many ways contraction of muscle, differs from it also in several respects. Two of these differences are rather striking and led BUCHTHAL, DEUTSCH, KNAPPEIS, and PETERSEN, as well as ASTBURY, PERRY, REED, and SPARK to the conclusion that "contraction" of actomyosin has little to do with muscular contraction. According to ASTBURY, "contraction" of actomyosin is simply a colloidal synaeresis while muscular contraction is an entirely different phenomenon. The two observations, on which this conclusion was based, were the following: muscle contracts anisodiametrically, becoming shorter and thicker without changing volume, while "contracting" actomyosin threads become shorter and proportionately thinner, thus simply shrinking. The second objection is based on BUCHTHAL's observation: while an unloaded actomyosin thread shortens in ATP, a loaded thread lengthens in the same solution, thus behaving contrary to muscle which shortens whether loaded or unloaded.

In this paper, the author, after pointing out certain analogies between the contraction of muscle and actomyosin, hopes to show that the objections raised by BUCHTHAL and ASTBURY can easily be explained and do not plead for a basic dissimilarity of the two processes.

If a washed fibre bundle of the *musculus psoas* of the rabbit is suspended in a Ringer solution, containing 0.001 M Mg and 0.2% ATP, it contracts and develops the same tension as the muscle developed maximally *in vivo*, showing that it was the normal mechanism of contraction which has been put into motion by ATP. This reaction is very specific, and all attempts to produce it with any other substance than ATP have hitherto failed. The same muscle fibre can be made to shorten also by other means, as for instance, by heat. At 70° shortening may be extensive, but no appreciable tension will be produced.

If the same washed psoas muscle is suspended in water and decomposed in the Waring blender into a suspension, on addition of the salts of the Ringer solution, a moderate flocculation will be observed. On addition of ATP an excessive precipitation occurs which has been termed "superprecipitation". Evidently, this superprecipitation

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is, in its essence, identical with muscular contraction, having been elicited by the same specific substance under a similar condition, the only difference being the destruction of the fibrillary architecture.

We can go one step further and dissolve out of the freshly minced psoas the contractile matter, actomyosin, by prolonged extraction by means of WEBER's alkaline 0.6 M KCl. This actomyosin behaves like the suspended muscle giving flocculation in presence of salts and superprecipitation in presence of ATP. The last step of degradation of the muscle may be the isolated extraction of actin and myosin. The two proteins, if mixed, unite to actomyosin which gives the same reactions as actomyosin extracted or the suspended psoas. This stepwise decomposition of the psoas thus gives identical results all the way, and the reactions, elicited by the highly specific ATP, are, in all phases, so similar that there can be little doubt about the essential identity of these reactions. Naturally, we must bear in mind that the fibril has its specific architecture which is present no more in suspensions.

Instead of making a suspension out of our actomyosin, we can also bring it into the form of a gel and make of this gel, by the method of WEBER, a fibre again. Suspended in pure water, the thread will swell. Addition of salts will make this swelling regress, a reaction which evidently corresponds to the flocculation of our actomyosin or muscle suspensions. On addition of ATP the thread, if thin enough, will shorten rapidly, a reaction which evidently corresponds to the superprecipitation of our suspensions and corresponds thus, also, in its essence, to contraction in muscle.

After having pointed out these analogies of actomyosin and muscle, let us consider the dissimilarities, quoted above.

Muscle shortens; actomyosin shrinks. This is certainly true, and our problem is whether this difference is due to a difference in the very essence of the reaction or whether it is due merely to the rough structural difference between fibril and actomyosin thread. In the former, as shown by the electron microscopic studies of HALL, JACUS, AND SCHMITT[8], the contractile filaments run all along the muscle fibril continuously, parallel to the axis. On extraction these filaments are broken up into fragments which are distributed at random in the actomyosin thread. If, in contracting muscle the filaments become shorter and wider, the muscle will have to do the same — become shorter and wider without changing volume. If the same shortening of filaments occur in the actomyosin thread which contains the fragments unoriented, at random distribution, the shortening of the very same filaments has to make the thread contract equally in all directions, that is make it shrink.

That this difference is actually due only to this difference in orientation can easily be shown. If the thread is gently stretched, as shown by GERENDAS, the filaments become oriented parallel to the axis similarly to muscle. If ATP is made to act on such an oriented thread, this thread will shorten and become wider, thus contract without changing volume, similarly to muscle. The same is true, as shown by BUCHTHAL and his associates after drying which acts as stretching.

PERRY, REED, ASTBURY, AND SPARK explain the "synaeresis" of actomyosin by a lateral association of particles. That this explanation cannot be correct is shown by the anisodiameteral contraction of the oriented actomyosin threads. In this structure the filaments are oriented parallel to the axis. Their lateral association could only make the thread thinner and never shorter, while the experiment shows that actually the opposite happens and the thread becomes shorter and wider.

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In order to be able to discuss the stretching of the loaded actomyosin thread in ATP, we have to give our attention for an instant to another effect of ATP, independent of contraction. Fresh muscle is elastic. Post mortem the ATP is disintegrated and, parallel to its disappearance, the muscle becomes inelastic, as shown by TH. ERDÖS, BATE-SMITH, AND BENDALL. It is possible to show that it was actually the disappearance of ATP which induced this difference. A washed psoas fibre is inelastic. If suspended in Ringer, containing ATP, it becomes elastic again. This shows that in absence of ATP, links are developed between neighbouring micells which make the system rigid, making slipping and relative motion impossible. These are abolished by ATP. This effect of ATP is independent of its second effect, contraction. If ATP did not have the first effect, it could not induce contraction at all because the system would be too rigid. This effect of ATP was, in fact, the very first specific effect discovered of ATP on "myosin" by ENGELHARDT, LJUBIMOWA, AND MEITINA who found that ATP makes "myosin" threads more extensible. The decrease of dynamic softness of actomyosin induced by ATP has also been studied extensively by BUCHTHAL and his associates.

After this short discussion we can consider now the extension of loaded actomyosin threads. If an actomyosin thread is loaded, it will not stretch because it is rigid, its particles being held together by the links or cohesive forces described before. If ATP is added these forces will be abolished and, under action of the load, the short fragments of filaments of which the thread is composed, will begin to slip under influence of the load, and the thread will lengthen, even if at the same time these fragments shorten. The situation will be different in an unloaded thread. There will be no force present to cause slipping, and the shortening micells will make the thread contract or "shrink" according to its co-axial or random distribution. In the muscle fibre there can be no slipping because the filaments run continuously through the fibrils, and so the muscle can shorten only if its filaments contract, whether loaded or unloaded.

PERRY, REED, ASTBURY, AND SPARK stress one more difference between muscular contraction and the contraction in actomyosin threads: the time factor. Muscle may contract several hundred times per second, while even thin threads need seconds for their contraction. Here again the difference lies in steric relations and not in principle. If diffusion and friction are eliminated, the ATP contraction is instantaneous. This can be shown in washed psoas-fibres suspended 0° C in a solution. At this temperature the fibres develop only a very weak tension. If they are transferred into a Ringer of, say 25° C, the development of a high tension is instantaneous. Rapid reaction can also be demonstrated in thin actomyosin threads, to which ATP is added in such a way as to reach the thread from one side. On this side the actomyosin contracts and makes the thread bend or curl up rapidly.

The differences in behaviour of muscle and actomyosin can thus, in the instances discussed, be explained satisfactorily by the rough structural differences of both formations and need not be ascribed to the difference in underlying reactions.

SUMMARY

It is shown that the contraction of muscle, superprecipitation of its suspensions, superprecipitation of actomyosin and contraction of actomyosin, elicited by ATP, are related phenomena.

Differences in behaviour, as for instance anisodiametry of shrinking in muscle and isodiametry of shrinking in unoriented actomyosin gels, can be explained by the differences in structure. The same is true for the difference of muscle and loaded actomyosin threads, the latter of which, contrary to muscle, lengthen under influence of ATP.

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RÉSUMÉ

Il a été montré que la contraction du muscle, la superprécipitation de ses suspensions, la superprécipitation de l'actomyosine et la contraction de l'actomyosine, provoquées par l'ATP, sont des phénomènes connexes.

Des différences de comportement, comme par exemple l'anisodiamétrie de rétrécissement du muscle et l'isodiamétrie de rétrécissement de gels d'actomyosine non orientée, peuvent être expliquées par les différences de structure. Le cas est le même en ce qui concerne la différence entre les fibres musculaires et les filaments d'actomyosine chargés, ces derniers s'allongeant, contrairement au muscle, sous l'influence de l'ATP.

ZUSAMMENFASSUNG

Es wird gezeigt, dass die Zusammenziehung des Muskels, die Super-Fällung seiner Suspensionen, die Super-Fällung von Aktomyosin und die Zusammenziehung von Aktomyosin, hervorgerufen durch ATP, mit einander zusammenhängende Erscheinungen sind.

Verschiedenheiten des Verhaltens, wie z.B. die Anisodiametrie des schrumpfenden Muskels und die Isodiametrie der Schrumpfung in unorientierten Aktomyosin-Gelen, können durch die Strukturverschiedenheiten erklärt werden. Dasselbe gilt für die Unterschiede zwischen Muskel und belasteten Aktomyosin-Fäden, welch letztere sich im Gegensatz zum Muskel unter der Einwirkung von ATP dehnen.

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It is a great pleasure and privilege to offer these lines to one of the most distinguished pioneers of muscle research; I wish him long years of undisturbed scientific activity.

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